The Tidal Rhythm of Emergence, and the Seasonal Variation of This Synchrony, in an Intertidal Midge

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Abstract. The emergence of an intertial midge was investigated at a site on the coast of the Inland Sea of Japan. The population emerging at this site was drawn from a single species of the genus *Clunio*, probably *C. tsushimensis*. Emergence was not synchronized with the day-night cycle, but with the tidal cycle. Moreover, the pattern of synchrony changed with season. A bimodal phase appeared in midwinter; but the pattern of synchrony shifted gradually, during January and February, from morning low tides to afternoon low tides, and a unimodal phase appeared in March. This pattern—*i.e.*, synchrony with afternoon low tides—lasted until early October. In mid-October, the synchrony shifted to the morning low tides. Only a brief bimodal phase appeared in autumn. The phase modality was clearly correlated with the height of tides; *i.e.*, when the low waters in a day were very different in height, emergence was synchronized only with the lower one (April to December). During January and February, the higher low tide, as well as the lower low tide, recedes considerably. The exposure of the larval habitat at the higher low tide may stimulate emergence, resulting in bimodal phases in midwinter. But the unimodal pattern in March cannot be accounted for by a simple synchrony with lower low tide, or with exposure of the larval habitat to the air; the day-night cycle not only would be one of the *zeitgebers* of the tidal rhythm in every season, but also must participate in the expression of the unimodal phase in spring. Furthermore, the number of midges that emerged each day fluctuated with a semilunar cycle with the season. The phase of this rhythm would be shifted by water temperature.

Introduction

Tidal and semilunar rhythms of reproductive activity have been reported in many intertidal and estuarine or-

ganisms (*e.g.*, see Korringa, 1947; Hauenschild, 1960; DeCoursey, 1983; Pearse, 1990). Marine midges of the genus *Clunio* are among such animals. The imagines emerge around the full and new moons, and the time of daily emergence corresponds to that of low tides at the habitat (Caspers, 1951; Koskinen, 1968; Hashimoto, 1976; Neumann, 1966; 1976; 1987). But in most of the species observed, eclosion occurs in a very short period, *i.e.*, at most the 4–5 days bracketing the full and new moons. In these cases, therefore, the rhythm underlying the observed events in the field—*i.e.*, daily or tidal—was not clear.

The circadian rhythm of terrestrial animals exhibits at least two essential and distinctive characteristics: (1) a free-running period approximating the 24-h day-night cycle; and (2) synchrony with the local light-dark cycle (and sometimes with 24-h temperature cycles, too). But in marine organisms, such characteristics are not sufficient evidence of a circadian rhythm. For example, the circatidal rhythm of larval release in a few semiterrestrial crabs is surely entrained by 24-h, light-dark cycles (Saigusa, 1986; 1992). We supposed that the emergence rhythm of *Clunio* might be similarly timed. As the first step in testing this hypothesis, the rhythm was examined in the field.

A further problem in tidal rhythm research is the relationship between the modulation of the tidal amplitude and the modality of the phase. In some of the oceans in the world, there are two high (or low) tides, of approximately equal height, per day, at intervals of about 12.4 h. But in the Pacific Ocean, the two high and low tides on a given day often show very different heights, and they recur at asymmetrical intervals (see Saigusa, 1985). Enright (1963) reported a circatidal rhythm of swimming in freshly collected specimens of the sand-beach amphipod *Synchelidium* sp. A feature of this rhythm was that the relative amplitudes of activity peaks seemed to reflect contemporary tidal amplitudes. An isopod, *Excirolana*
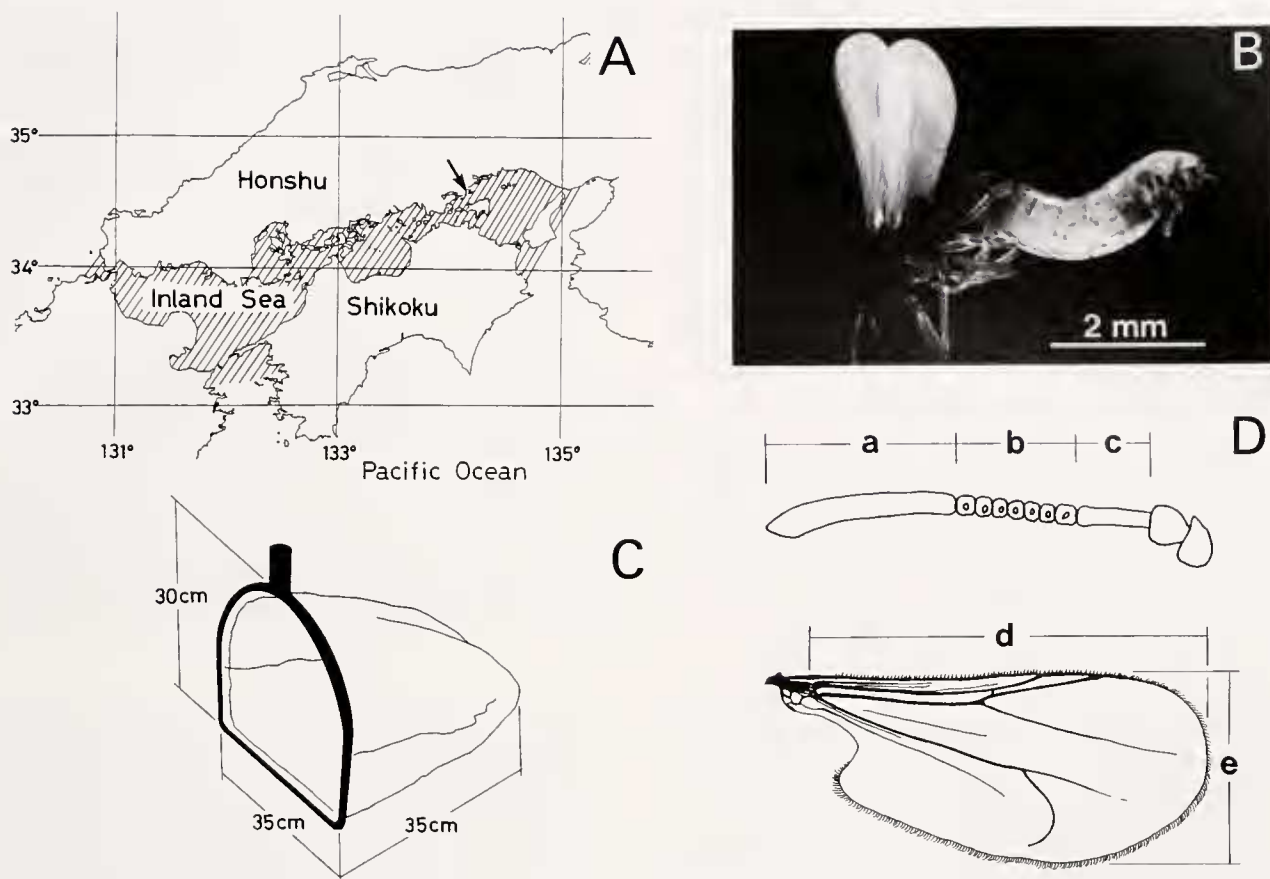
Figure 1. (*A*) The site of emergence of the *Clunio* population studied (arrow). The Inland Sea is situated between Honshu and Shikoku (shown by the diagonal lines). As its east and west sides are open to the Pacific Ocean through the straits, the salinity is not greatly reduced, averaging 28–29 ppt through the year. (*B*) Recently emerged male and female imagines shown mating. The pupal eclosion of the female is completed with the assistance of the male. As soon as he has stripped off the female's pupal skin, the male runs or flies carrying the female in the mating position shown in this figure. (*C*) The nylon net used to catch flying midges. Since many midges fly on the water film, the sampling was carried out with the net touching the water. (*D*) Antenna and wing venation of a male *Clunio* studied: *a*, the third segment; *b*, adjoining seven small segments; *c*, the ultimate segment; *d*, length of the wing; *e*, width of the length. See the text for the antennal and wing indices.

chiltoni, inhabiting a similar environment exhibited a similar activity rhythm (Enright, 1972).

Tidal amplitudes vary with the season. Thus, if the activity of an organism is correlated with tidal amplitude, then the pattern of the rhythm must also vary with the season. We have already reported on the swimming activity rhythm of the sublittoral crustacean *Dimorphostylis asiatica,* whose pattern was modified seasonally (Akiyama and Yoshida, 1990). The midge *Clunio* is also an appropriate animal with which to investigate this problem, because emergence occurs in every season. Oka and Hashimoto (1959) reported a seasonal change in daily emergence in *Clunio tsushimensis.* But their data were only qualitative (see also Hashimoto, 1976) so it was not clear whether the daily timing of emergence is correlated with

modulations of tidal amplitude or with other seasonal factors, such as the ratio of day-night length or the fluctuation of water temperature.

An additional problem is the uncertain classification of the genus of midges and the distribution of species along Japanese seacoasts. Hashimoto (1969, 1976) reported a sympatric species, *C. aquilonius,* whose morphology is quite similar to that of *C. tsushimensis.* So we had to identify the species whose imagines were emerging in our study area along the shoreline of the Inland Sea.

Materials and Methods

Collections of flying midges

Field investigations were carried out on the coast of the Inland Sea of Japan, Okayama Prefecture (Fig. 1A);

the exact site was the rocky seashore in front of the Ushi-mado Marine Laboratory. The Inland Sea is bounded to the south by Shikoku and is connected to the Pacific Ocean through straits at both sides of Shikoku. Thus, heavy waves do not roll ashore except when there are strong winds. The tidal amplitude varies between −15 cm (minimum level of low waters in the tide table published by the Japan Meteorological Agency) and 260 cm (maximum in high waters) during the year. The tidal pattern is semidiurnal, but morning low waters recede much further than afternoon low waters in winter, and the pattern is reversed in summer.

Male imagines fly or swarm immediately after eclosion. They are most abundant within a narrow area—a strip about 3–4 m wide along the water line of the rocky shore. Outside this narrow strip, the number of flies rapidly decreases. The emerged males scurry about on the rocks or stones while vigorously vibrating their wings or they skim rapidly over the water. When the male finds a female pupa, he strips off her pupal cuticle and copulates with her. The female (Fig. 1B) soon lays eggs on the rocky substrate. The females are always much less numerous than the males—less than 10% of the males emerging at the same time. In any event, when the tide starts to rise, the emerged adult midges all die.

We collected the adult males by sweeping the surface of the water with a nylon net (Fig. 1C). At the beginning of the emergence, the midges were seen only on the surface of the water, but around the time of low tide, large numbers of males were seen on the rocks as well as on the water surface. But it was hard to collect them on the rocks without some type of suction device, so the surface water was scooped up to capture the midges skimming over the water.

The sweeping was carried out on the rocks for 5 min every 30 min: the sampling range was 1–1.5 m along the water's edge (220–240 strokes per 5-min sampling period). Most emergence occurred within the 4–5 h surrounding the time of low water, and the water receded, at most, 50 cm during the period, *i.e.*, the time between the start and end of emergence. Therefore, we were not required to move the collection site frequently as the tide declined; we always swept only two rocks that differed by about 50 cm in height. The samples were quickly brought to the laboratory and transferred to a pail containing warm water (20–30°C). The midges rose to the surface of this water and were picked up with a forceps and counted.

We needed to determine whether the midges emerge and fly at the time of high tides as well as low. In the daytime, flying midges can be seen from the shore, but if only a few specimens were flying on the water surface, we might overlook them, especially at night. To eliminate this possibility, we used a light for some collections. Like other insects, marine midges are attracted to light at night.

The light (180 W) was placed at the edge of a floating pier (5–6 m long), and the midges that swarmed around this spot were collected by sweeping only two strokes with the net. This method—collection under the light—was used only for the initial and preliminary observations, and the resulting data about emergence at high tide are only in Figure 4A. All other data were obtained by sweeping without lighting.

Investigations of the larval habitat

The habitat of the larvae with respect to the level of tides was examined. While the tide was ebbing, the algae growing on the rock were taken along with their associated sand or mud. These substrata (36 cm²) were removed from various heights within the intertidal zone and transferred, in the laboratory, to a vessel containing seawater. When the vessel was shaken by hand, most larvae escaped from the nest tubes buried in the sand or mud. Each larva was picked up by forceps and counted. These samples were taken on 21 and 31 March 1991.

Identification of the species emerging

The taxonomic classification of the genus *Clunio* is not easy because definitive morphological differences are lacking. Hashimoto (1969) reported a sympatric species, *C. aquilonius*, that is extremely similar to *C. tsushimensis* and also occurs along the coasts of Japan. The two species can be distinguished on the basis of three morphological properties: antennal index, wing index, and body length. The antennal index is determined on the 11-segmented antennae of the male: *i.e.*, the ratio of the length of the ultimate segment (c) to the length of the 3rd to 10th segments (a + b) (Fig. 1D). The wing index is the ratio of the width (e) to the length (d) of the wing (Fig. 1D, and Hashimoto, 1968, 1969).

It was critical for us to determine whether both species are sympatric along the coasts of the Inland Sea, because different species might emerge during the day and night. Therefore, specimens were compared under a stereomicroscope, not only for the characters described by Hashimoto (1968, 1969), but also for their emergence during morning and afternoon low tides.

Results

Morphology of the Inland Sea population and larval habitat

To determine whether a single species of *Clunio* emerges during both the morning and evening low tides, we compared the antennal and wing indexes and the body length of specimens collected at those times. As shown in Figure 2A, the distribution of the antennal index (AI) was almost the same whether the specimens were collected at morning
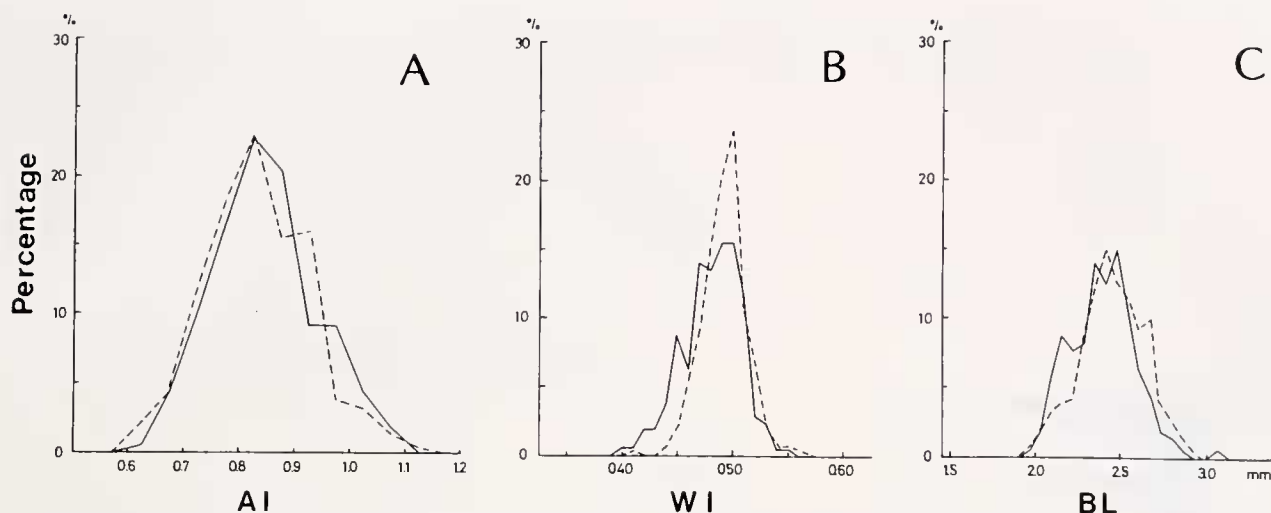
Figure 2. Comparison of the distribution of antennal index (AI), wing index (WI), and body length (BL) among the male *Clunio* population. Date of collection: 30 March 1991. Solid lines: specimens collected at the afternoon low water. Broken lines: those collected at the morning low water. Total number of the specimens measured: about 250 individuals at each tide.

low tides (mean 0.83) or afternoon low tides (mean 0.85). The wing index (WI) was also much the same (mean 0.49 and 0.48, respectively) among the two groups (Fig. 2B). Furthermore, as shown in Figure 2C, the mean body length (BL) was also much the same for specimens emerging in morning and afternoon (2.43 mm and 2.37 mm, respectively). The lack of a clear difference between the specimens collected at the morning and afternoon low waters suggests that the population of *Clunio* emerging in this location is a single species.

Figure 3 summarizes the distribution of the larvae with respect to the tidal height. The larvae of *Clunio* preferred the thin, feltlike substratum found in shallow hollows on the rocks; this substratum consisted of filiform green algae and sandy mud. The highest larval densities were recorded at 70–110 cm, and the fewest larvae were at the sites lower than 50 cm.

On this rocky shore (Fig. 1A), *Spaniotoma nemalione* (Subfamily Orthocladinae) was also abundant in March. Imagines of this species were easily distinguished from those of *Clunio:* their bodies were much slimmer, they had a different swarming site (*i.e.,* above oysters exposed to the air), and their way of flying was different. Although we could not specifically identify the larvae of this species, the difference in the swarming site would suggest that *Spaniotoma* larvae were not mixed in the substrata samples.

Tidal rhythm of emergence and its pattern in winter

To determine the relationship between the timing of emergence and the tidal cycles, collections were made through the night. As shown in Figure 4A, emergence did not occur at the time of high tide. Emergence and swarming started a few hours before the time of low tide and had ceased by 3–5 hours after. The pattern of emergence was clearly that of a tidal rhythm, and not that of a daily rhythm. Another feature of this emergence rhythm is the semilunar timing; *i.e.,* the fluctuation in the number of midges emerging every day is correlated with the lunar cycle. But the peaks of the semilunar rhythm did not accurately coincide with the days of full and new moons; in the data of Figure 4A, they occurred a few days before the full and new moons, respectively (see also Fig. 7b).

Figure 4B indicates the time of emergence from January to February. Emergence is clearly synchronized with the low tides. Moreover, the phase of the tidal rhythm is bimodal; i.e., the timing is clearly synchronized with *both* of the low tides that occur each day. A further aspect of this rhythm is a modulation of the tidal synchrony. As noticed from the data of the first 17 days (*i.e.,* 9–25 January), the midges emerging during the afternoon low tides are outnumbered by those emerging during the morning low tides. During the next 14 days (26 January–8 February), the number of midges emerging in synchrony with the morning low tides is about equivalent to the number emerging during the evening low tides. During the following 20 days in February (Figs. 4B and 5A), the midges emerging at the morning low tides are outnumbered by those emerging at the afternoon low tides.

The pattern of emergence in spring and summer

In March, the synchrony with the afternoon low waters is remarkable. As shown in Figure 5A, very few midges
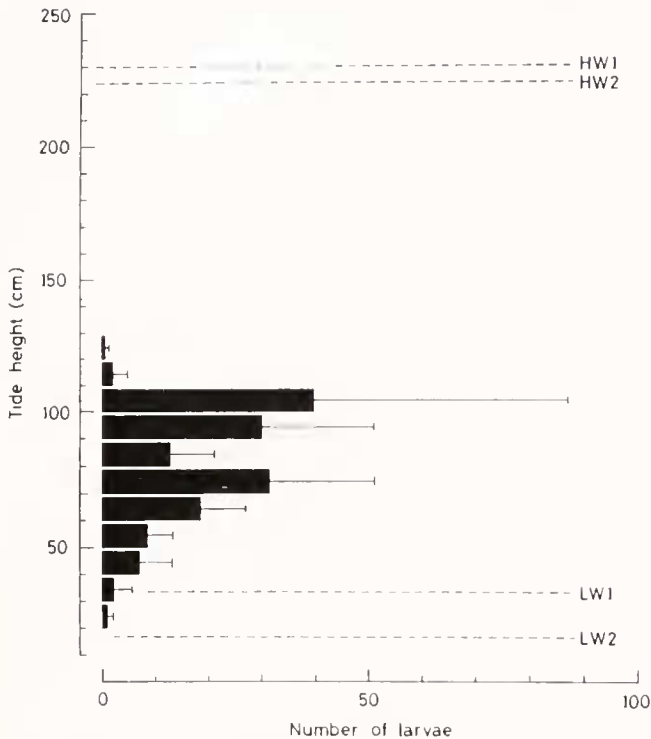
Figure 3. Relationship between the tidal height and the density of the larvae. Thick horizontal bars indicate the mean of the number of the larvae at each height; the error bars show the standard deviations; 74 samples were collected. *HW1, HW2* and *LW1, LW2* show the two high and low tides about the time of sampling, respectively. The height is based on the data in the tide table published by the Japan Meterological Agency.

emerged in synchrony with the morning low tides (see the morning on 13, 14, 16, 17, and 27–30 March); a great majority of the animals were collected during the afternoon low tides. Another feature is that the number of midges emerging in March and April increased drastically with respect to the population observed in January and February (compare the data of Fig. 7b and 7c).

After April, the timing in synchrony with the early morning low tides completely disappeared. Therefore, the tidal rhythm clearly shows a unimodal phase (Fig. 5A and 5B). Although field observations were not made frequently during the early morning low tides from May to September, it is clear that no midges emerged at these times; rather all midges emerged during the midday and afternoon low tides (Fig. 5B, 5C, and unpub. data). Comparison of the rhythmic patterns shown in Figure 5, A–D demonstrates that the time of emergence advances from spring to summer, with respect to the 24-h day-night cycle. In Figure 5A, emergence was observed around sunset; *i.e.,* 1600–2100 in early March, 1400–2000 around the new moon on 16 March, and 1300–1800 around the full moon on 30 March. In April and May (Fig. 5B), emer-

gence occurs between noon and sunset. It advances a few hours further in June and July, when most midges appeared between 1000 and 1600 (Fig. 5C). In August and September, emergence occurs at midday (Fig. 5D). In every case, the daily timing of emergence is clearly synchronized with the times of low tides. If we were to assume a circadian rhythm underlying the *Clunio* emergence rhythm, then we would have to consider a drastic phase-advance of the rhythm from March to September. Such an assumption is not reasonable; these results must be interpreted in terms of an expression of the tidal rhythm corresponding to a phase-advance of the peak of the semilunar rhythm (see Fig. 7c–7f).

Alteration of synchrony in autumn

The unimodal tidal pattern was synchronized with the daytime low tides until September (Fig. 5, A–D). But in the latter half of October (Fig. 6A), the synchrony of timing was altered from daytime low tides to nighttime low tides. Most midges emerge in synchrony with the low tides at night in November (Fig. 6A) and December (Fig. 6B). Unlike the winter population (Fig. 4B), the autumnal emergence shows no clear bimodal phase (see also Fig. 9). A feature of the autumnal tidal rhythm is that the daytime emergence occurs only for the first several days of the fortnightly peak of emergence (*i.e.,* 17–22 October, 30 October–2 November, and 16–17 November in Fig. 6A; and 30 November–1 December, and 14–16 December in Fig. 6B).

Seasonal fluctuations in the number of midges emerging, semilunar timing, and a seasonal shift of the peak

Figure 7 summarizes the fluctuations in the number of flies emerging every day in relation to the lunar cycle. The number of collected midges is extremely small in January and February (Fig. 7b); but it suddenly increases thereafter. A large number of the midges were collected in March and early April (Fig. 7c). In late April and May (Fig. 7d), the number of emerging midges decreases. Not many midges emerge in June and July (Fig. 7e), and the number of midges is very small in August, September (Fig. 7f), and early October (Fig. 7g), reaching a maximum in November (Fig. 7h). The number of midges emerging every day thus fluctuates seasonally with two peaks: one in March–April, and another in November. Moreover, very few midges emerge in January–February and August–September.

Another feature of seasonal fluctuation is the semilunar periodicity. But the peak of this rhythmicity shifts several days in relation to the lunar cycle. In February and March (Fig. 7b, 7c), it occurs 2–4 days after the full and new moon; it just coincides with the days of full moon in April
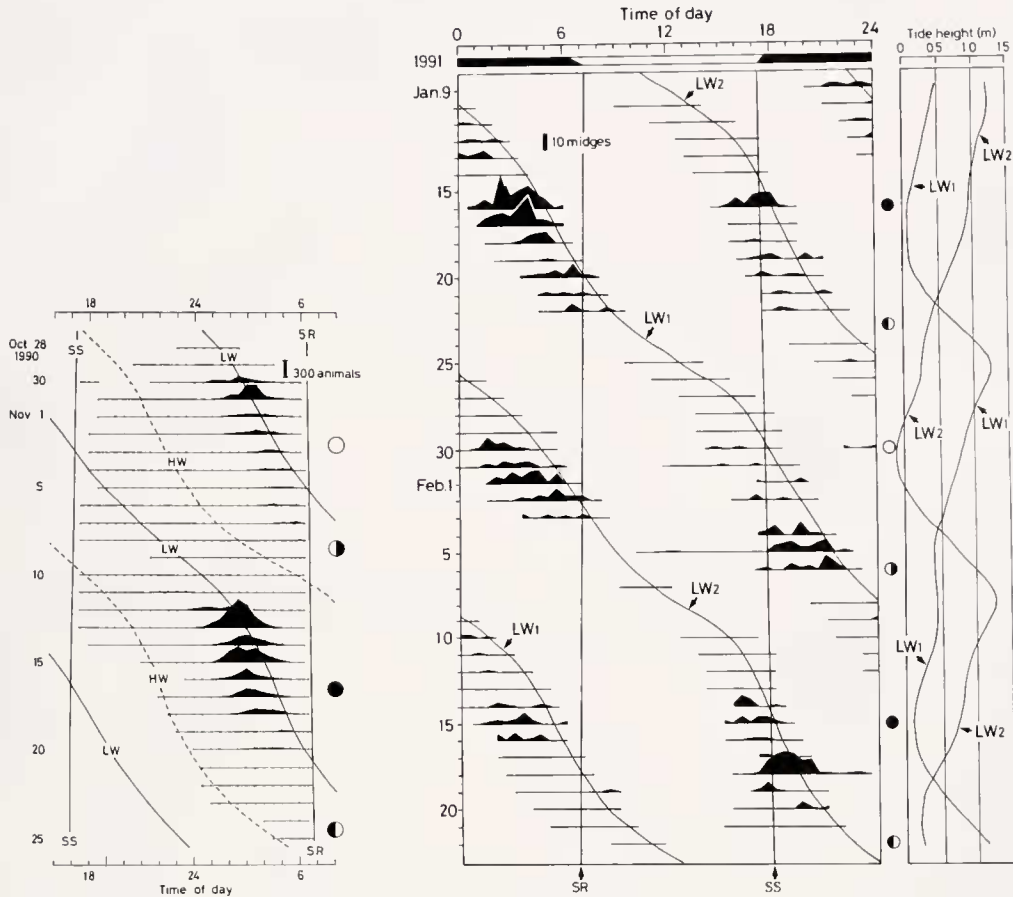
Figure 4. Daily timing of emergence of *Clunio tsushimensis* males in relation to day-night, tidal, and lunar cycles. (Left) The record from 28 October to 25 November 1990. Midges were collected using a light trap method. The number of midges is plotted vertically on the horizontal lines, which also show the 5-min period every 30 min (dot) during which sampling was done. Absence of the horizontal bars indicates no sampling. SS and SR represent the times of sunset and sunrise, respectively. *HW* and *LW* connect the respective times of high tides and low tides at the habitat. Open circle: full moon; black circle: new moon; semicircles: the first and last quarters of the moon. The vertical scale indicates 300 midges. (Right) Wide left panel: the pattern of emergence in midwinter (the record from 9 January to 22 February 1991). Midges were collected by sweeping for 5 min every 30 min. The number of midges is plotted vertically on the horizontal line during which the sampling was made. The vertical scale shows 10 midges. *LW1* and *LW2* connect the times of low tides at the habitat. Other symbols are as in Figure 4A. Narrow right panel: fluctuations in the height of the two low tides, *LW1* and *LW2*.

(Fig. 7c, 7d). The peak then advances from the days of full and new moon: in June and July (Fig. 7e), it appears 4 days before the syzygy, and from August to October (Fig. 7f, 7g), it advances near the half moon. The peak is again close to the full and new moon from November to December (Fig. 7h).

To examine the relationship between the shift of the semilunar peak and the temperature of the habitat, each peak of the semilunar rhythm was plotted in relation to the seasonal fluctuation of water temperature. As shown in Figure 8, the peak (*i.e.*, the mean of the distribution of emerging midges in each semilunar cycle) is represented by the days shifted from each full and new moon. The

water temperature is expressed by the average values of 15 days at each month. The shift of the peak from the days of full and new moon clearly coincides with the fluctuations of water temperature in the habitat, suggesting that the phase of the semilunar rhythm is influenced by the temperature.

Discussion

The emergence of *Clunio tsushimensis* imagines has a clear tidal rhythm. Moreover, the pattern of synchrony changed with season. A bimodal phase appeared in midwinter; but the synchrony shifted gradually, during Jan-
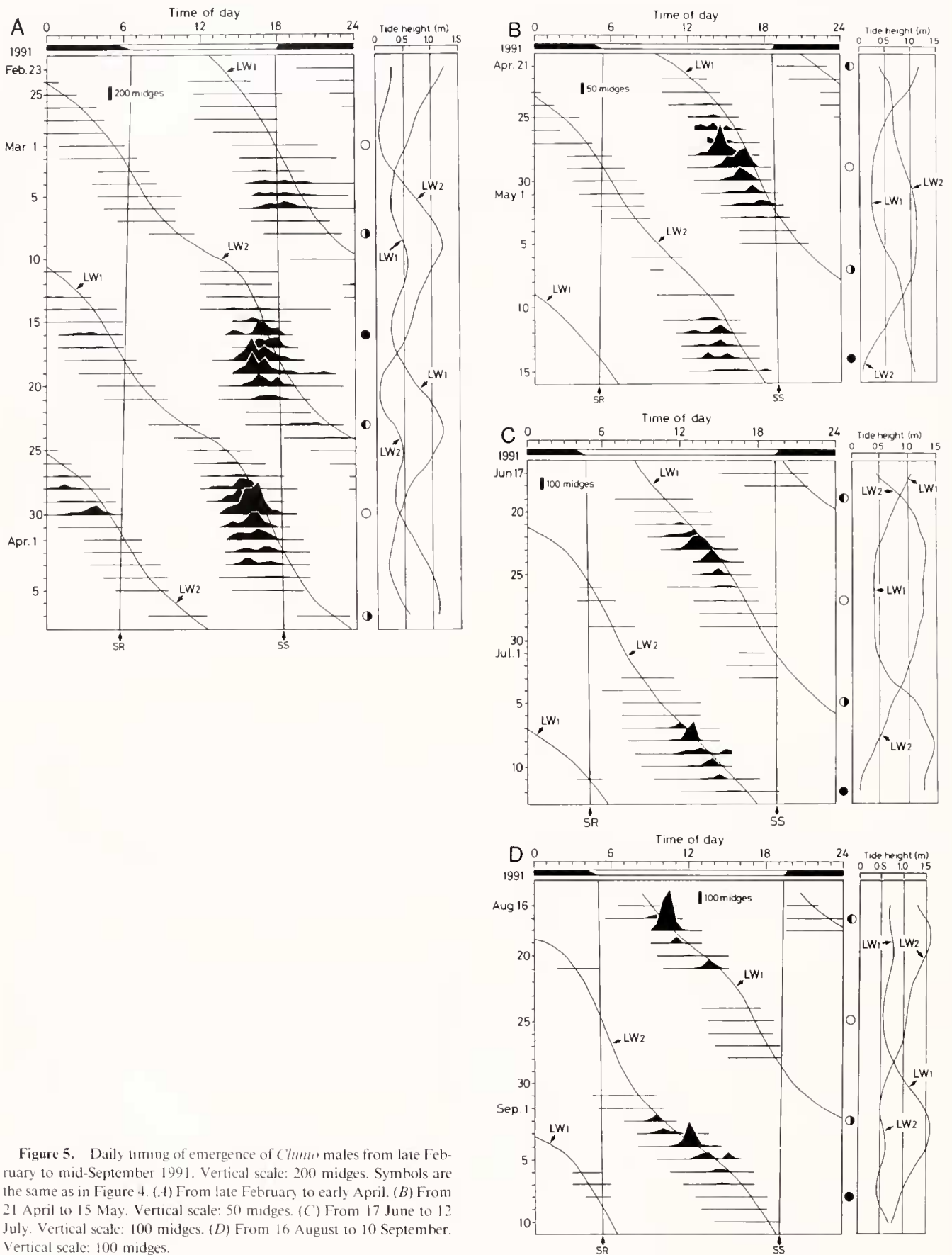
Figure 5. Daily timing of emergence of *Clunio* males from late February to mid-September 1991. Vertical scale: 200 midges. Symbols are the same as in Figure 4. (*A*) From late February to early April. (*B*) From 21 April to 15 May. Vertical scale: 50 midges. (*C*) From 17 June to 12 July. Vertical scale: 100 midges. (*D*) From 16 August to 10 September. Vertical scale: 100 midges.
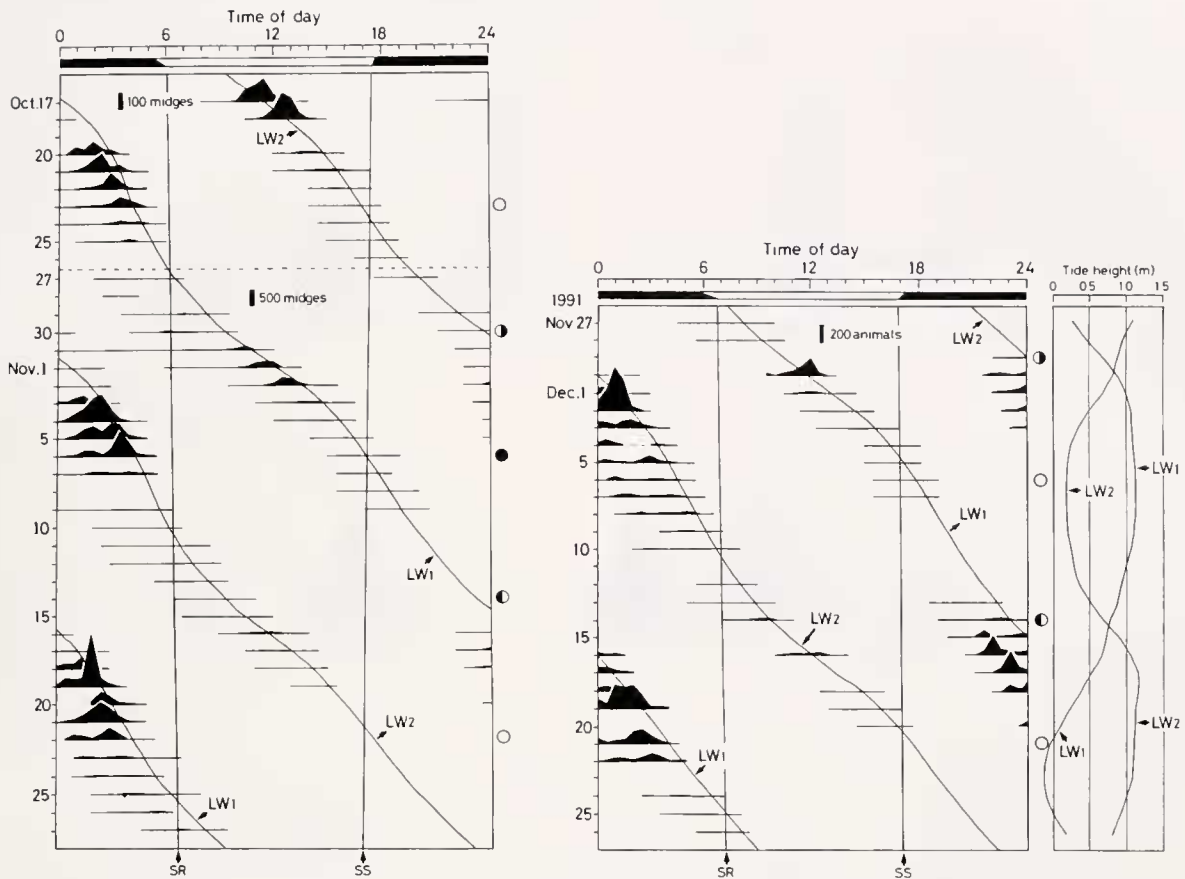
Figure 6. Daily timing of emergence of *Clunio* males from mid-October to the end of December 1991. Symbols are the same as in Figure 4. (Left) In autumn (17 October–27 November). The number of emerged midges drastically increased from October to November (compare Fig. 7g and 7h), so the figure was separated into two panels (horizontal dashed lines) with different vertical scales. The data on emergence in this period are shown in duplicate in Figure 9, with the fluctuations of the two low tides (*LW1* and *LW2*). (Right) From 27 November to 26 December. Vertical scale: 200 midges.

uary and February, from morning low tides to afternoon low tides, and a unimodal tidal rhythm in synchrony with afternoon low tides appeared in March. This pattern lasted until early October. In mid-October, the synchrony shifted to the morning low tides. As a result, emergence was observed during the day from spring to autumn, at night in early winter, and in both daytime and nighttime in midwinter. Therefore, we must ask a question about the modulation of the expression of that rhythm: What are the factors that induce the expression of the bimodal pattern and that cause one of the two low tides to synchronize the timing?

Relations between the tidal patterns and the phase expression of the tidal rhythm

On most Japanese seacoasts fronting the Pacific Ocean, there are two high and two low tides per day, at mean intervals of 12.4 h; the amplitude of these tides changes with a fortnightly periodicity, resulting in spring and neap tides. But the amplitude of these tides on a given day varies with season. For example, on 16 January (new moon; see Fig. 4B), a *low* low tide (+0.09 m) occurs at 0500. A *high* high tide (+2.3 m) is followed after 7 h by this low tide, which is then followed after 6 h by a *high* low tide (+0.9 m), and further followed after 5 h by a *low* high tide (+1.9 m). The height of the two low tides becomes similar 6 days after the new moon. Thus, from late autumn to winter, a *low* low tide always occurs in the morning, and a *high* low tide always occurs in the afternoon (Figs. 4B, 6B, and 9). In contrast, as shown in Figure 5, B–D, a *low* low tide always occurs in the afternoon, and a *high* low tide occurs in the morning during spring and summer.

A similar complex tidal regime is seen on the Pacific coast of North America. Enright (1963, 1972) recorded the swimming activity of several kinds of crustaceans that were freshly collected from the sandy beach of Cali-
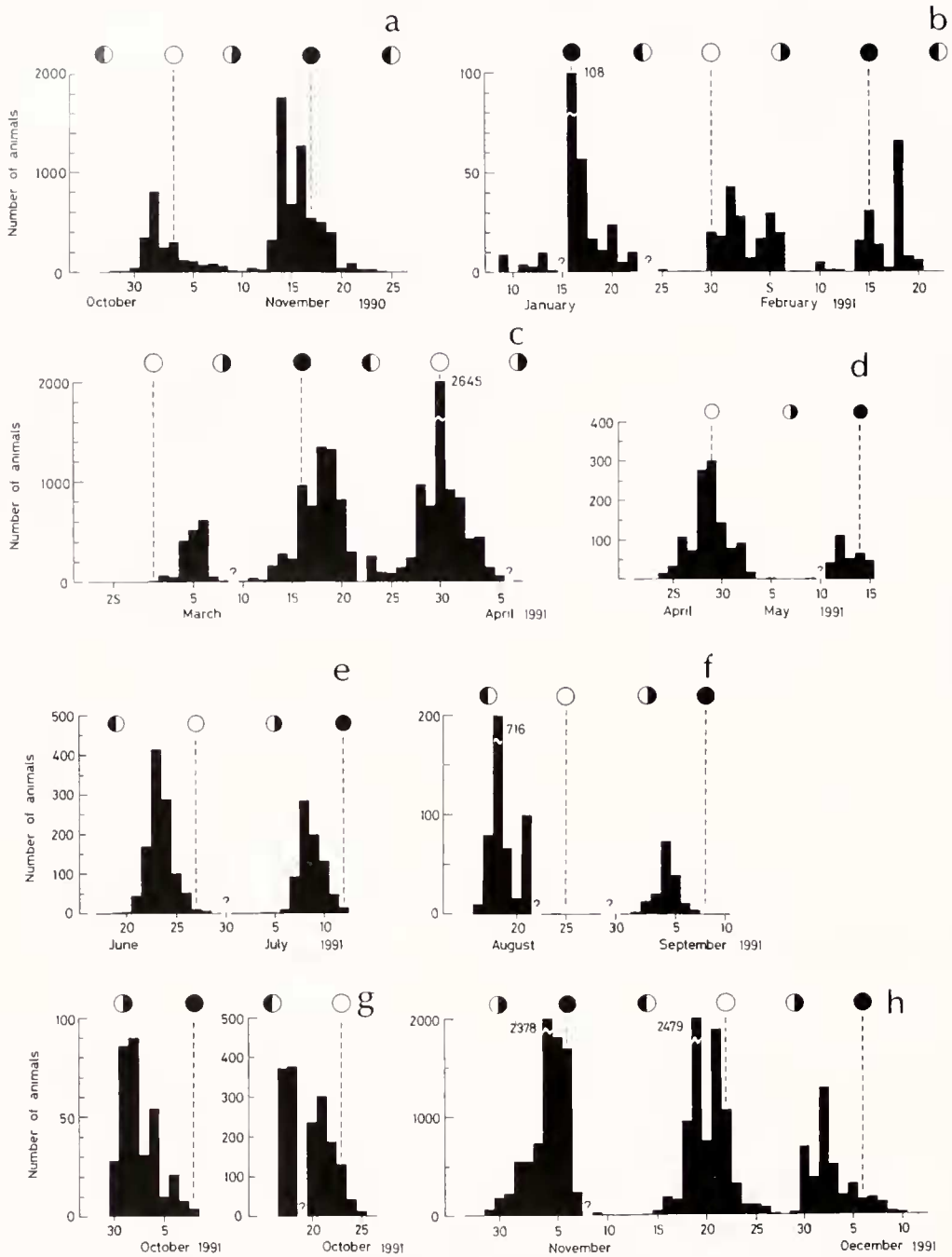
Figure 7. Semilunar rhythm of emergence of *Clunio tsushimensis* males, and the seasonal fluctuation of the number emerging every day. The vertical black bars indicate the number collected per day in relation to lunar cycles. Open circles: full moon; dark circles: new moon; semicircles: the first and last quarters of the moon. Question marks on the horizontal axis indicate days when the sampling was not made.

fornia. The activity rhythm of the amphipod *Synchelidium* sp. was progressively damped as the animal's time in the laboratory was prolonged, but it was well defined for the first few days after collection. Another feature of the activity pattern is that the relative amplitude of its peaks reflects the amplitude of the tides occurring at the nearby seacoast (Enright, 1963). Similar activity rhythms were also recorded from the isopod *Excirolana chiltoni* (Enright, 1972). This animal showed a persistent tidal rhythm for 2 months under the constant conditions in the laboratory. The temporal variation in activity seems to parallel predicted changes in the height of high tide.
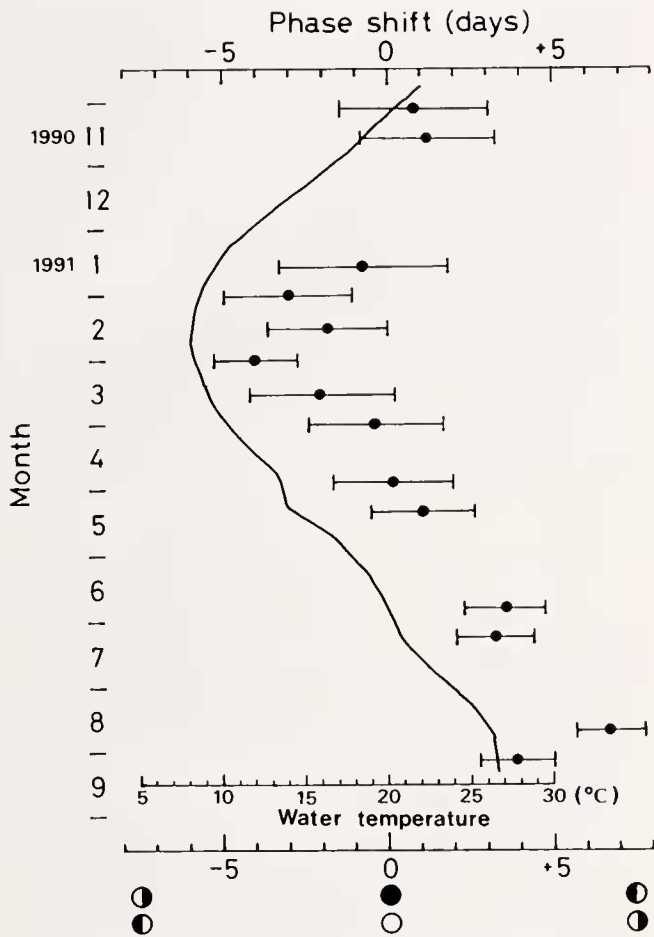
Figure 8. The relationship between the phase of the semilunar rhythm and the seasonal change of the mean water temperature at the habitat (solid curve). Each peak (black circles) of the semilunar rhythm is indicated as the days of shift from full or new moon (*i.e.*, 'zero' on the horizontal axis). The error bars indicate a standard deviation within each peak of the semilunar rhythm. The peaks on the right of 'zero' show a phase-advance of the semilunar rhythm with respect to the syzygy, and those on the left of 'zero' show a phase-delay from the syzygy.

To examine whether the tidal synchrony of the rhythm of emergence of *C. tsushimensis* could be altered by irregularities in the amplitude of the semidiurnal tides in the habitat, the water level of low tides was plotted against the time of emergence. Through the autumn, as shown in Figure 9, the relative heights of the two low tides—*i.e.*, the *low* low tide and *high* low tide—vary continuously, but cyclically, with an interval of 2 weeks. When the two tides were different in amplitude, emergence clearly coincided with *low* low tides alone, which produced the unimodal phase of the tidal rhythm. A bimodal phase appeared for only a few days every fortnight when the heights of the low tides were close to equal.

Similar relations are also seen in December, when emergence occurred in synchrony with the *low* low tides

(*i.e.*, the nighttime low tides), causing a unimodal phase except a few days around the half moon (Fig. 6B). The tidal pattern in January (Fig. 4B) somewhat changes from that of October–December (Figs. 6B and 9): while the height of the *high* low tide exceeds 1 m on many days in December (Fig. 6B), it is less than 1 m on most days in January (Fig. 4B). Therefore, in January, the main part of the larval habitat (see Fig. 3) is exposed to the air two times per day, except a few days after the half moon. The decrease in height of the *high* low tide might influence the larval habitat, which might have evoked an emergence, resulting in bimodal phases during a relatively long period in January and February.

The correlation between the relative difference in the tidal heights and phase modality was noticed no later than the first 10 days of March; thereafter it was lost. In March, for example, the height of both low tides is close to equal around the full and new moons, yet far more midges emerged in the afternoon than in the morning (Fig. 5A). This result cannot be accounted for by a simple synchrony with *low* low tide, or by the exposure of the larval habitat to the air at low tides. Day-night cycles must participate in the selection of the afternoon low tides. Thus, the synchrony of emergence with the two low tides per day is correlated, not only with the relative difference in the heights of the tides, but possibly also with the day-night cycle.

In May, the timing of emergence is again synchronized with the lowest of two different low tides, and a unimodal phase appears in the daytime (Fig. 5B). During June and September (Fig. 5C and 5D), neither of the two low tides ebbs less than those of spring. In each case the timing of emergence is synchronized with the *low* low tides, and a unimodal tidal rhythm appears. Since the *low* low tides occur in the daytime from the end of April to September, the unimodal phase also appears in the daytime.

The relationship between the *low* low tides and the 24-h day-night cycle is reversed in autumn. As shown in Figure 9, the *low* low tides appear in the morning. Emergence is synchronized with the *low* low water, and shows a unimodal tidal rhythm. As a result, the phase of this tidal rhythm appears in the morning. Moreover, the *high* low tides occur in the afternoon, and do not ebb lower than 0.8–0.9 m. If the larval habitat is not moved in height from that of March (Fig. 3), there are only a few days every 2 weeks when the habitat is exposed to the air at each low tide (*i.e.*, twice a day). This might have resulted in a brief bimodal phase in autumn and early winter (Fig. 6A and 6B).

Zeitgeber *of* Clunio *tidal and semilunar rhythm*

Few cyclical environmental factors are known to be *zeitgebers* of tidal rhythms. One such is the cycle of water
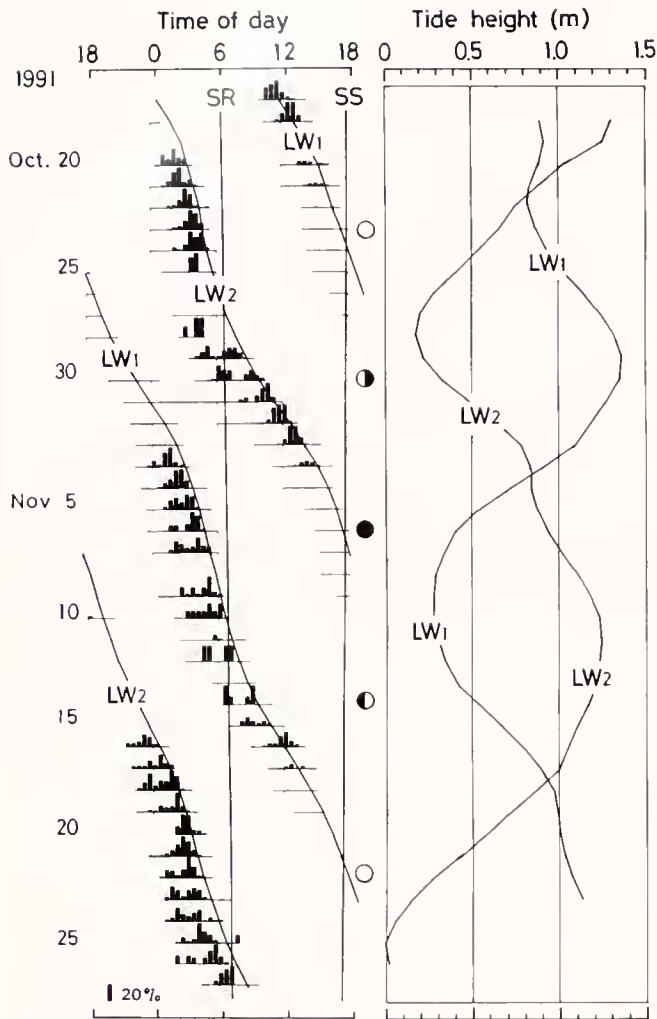
Figure 9. The relationship between the daily timing of emergence and the heights, on the same day, of two low tides ($LW1$ and $LW2$). The data on emergence are the same as in Figure 6A. To make that relationship much clearer than in Figure 6A, the numbers of emerged midges are shown as a percentage of the total number collected per day. The vertical bar shows the scale of 20%. The right panel shows the fluctuation of the heights of the two low tides ($LW1$ and $LW2$).

controlled by a circadian clock that is entrained by a 24-h day-night cycle only. But the phase of the tidal cycle differs according to the habitat of animals (*e.g.*, see Fig. 9 in Saigusa, 1988). Neumann's hypothesis cannot explain why the phase of the daily emergence rhythm coincides with the time of low tide in each larval habitat. Moreover, as indicated by this study, the phase of the daily emergence cycle was largely shifted through the year, with respect to the 24-h day-night cycle. Such a large phase-shift could be explained in terms of the tidal rhythm.

In crab larval release activity (Saigusa, 1986), the 24-h day-night cycle causes the phase shift of the tidal rhythm with a unimodal phase. Similarly, as shown in other species (Saigusa, 1992), this factor also causes the phase shift of the bimodal tidal rhythm. Thus, it is clear that the 24-h day-night cycle can be one of the *zeitgebers* of circatidal rhythms.

But it would be impossible that the tidal rhythms are entrained by the 24-h day-night cycle alone; other factors correlated with the tidal cycle, such as water turbulence, must be considered. Moreover, moonlight cycles in parallel with the tidal cycle could be the candidate, although the phase angles of these two environmental cycles differ according to geographical conditions. Cycles of artificial moonlight do entrain the tidal rhythm of larval release in an estuarine terrestrial crab; and the phase relations between the evoked tidal rhythm and the artificial moonlight cycle correspond to the phase relations between the time of high tide and the moonlight cycle in the habitat of a local population (Saigusa, 1988).

As this study indicates, the timing of emergence is synchronized with the time of low tides and shows a tidal rhythm through the year. So we can speculate that among the possible *zeitgebers* of the *Clunio* tidal rhythm is the ebb-flow cycle of the tides, the moonlight cycle, or both. In addition, as suggested from the experiments with *Clunio* (Neumann, 1966, 1976) and crabs (Saigusa, 1986, 1992), the 24-h day-night cycle could also be the *zeitgeber* of the *Clunio tsushimensis* tidal rhythm. Furthermore, while the day-night cycle entrains the tidal rhythm in every season, it would be involved in the expression of a unimodal phase (see Fig. 5A).

A problem in understanding tidal rhythms having a semilunar component

Enright (1972) reported endogenous swimming activity rhythm of *Excirolana chiltoni* inhabiting the sandy beaches of the Pacific coast of North America. The tidal regime in this habitat includes semidiurnal inequality in amplitude. The tidal scheme recurs at double-tidal intervals (*i.e.*, 24.8 h), which further changes at intervals of 2 weeks. *E. chiltoni* shows a persistent circatidal rhythm with a lunar component under constant conditions in the

turbulence (Enright, 1965). Cyclical or noncyclical changes in hydrostatic pressure also cause behavioral responses in amphipods (Enright, 1962; Morgan, 1965). Hydrostatic pressure fluctuations do not entrain an 'endogenous' rhythm, but since they coincide with the tidal cycles in the field, they could be the *zeitgeber* of the tidal rhythm. But, at least for the swimming activity of intertidal isopods, the 24-h day-night cycle does not seem to be a *zeitgeber* of tidal rhythms (Enright, 1963).

In *Clunio* emergence rhythm, Neumann (1966, 1976) showed that the semilunar timing is entrained by the artificial moonlight given in the laboratory for 3-4 nights every 30 days. According to Neumann's hypothesis (Neumann, 1976, 1985, 1987), daily timing of emergence is

laboratory; even under such conditions, activity reflects different heights of the tides (see Fig. 1 in Enright, 1972). Nevertheless, if the activity were plotted on a 24-h time scale, the rhythm would appear to be circadian.

Similar phenomena occur in *Clunio tsushimensis,* as we have reported here. The tidal rhythm of this animal showed a unimodal phase except in January and February. Furthermore, this tidal rhythm features a semilunar timing, with emergence occurring for several days near the times of the full and new moon (Fig. 7). From March to September, emergence is synchronized with the afternoon low tides (Fig. 5A–5D). If the data for these periods are plotted against the time of emergence, a daily rhythm with a peak at the afternoon appears (*e.g.,* Fig. 10A). On the other hand, from November to December, emergence occurs at the time of morning low tides (Fig. 6A and 6B). Now if the timing is plotted, a daily rhythm appears with a peak in the morning (Fig. 10B).

The emergence of *Clunio* is concentrated around the times of the full and new moon. So, if the data were arranged as shown in Figure 10, we could not readily determine whether the rhythm that underlies the daily and semilunar cycle of emergence is tidally correlated or day-night correlated. Yet, as stated repeatedly, the daily timing of emergence is not explicable in terms of a circadian rhythm or a modified circadian rhythm. It would be a tidally correlated rhythm that underlies the expression of a semilunar rhythm. Moreover, this rhythm is surely entrained by a 24-h, day-night cycle. Nevertheless, there is no evidence that the two internal rhythmic systems—circadian and circatidal rhythms—couple or interact together, resulting in a semilunar rhythm (Saigusa, 1986, 1988, 1992). These problems bear on the timing mechanism of tidal and semilunar rhythms and require further consideration.

A local population of Clunio tsushimensis

The systematics of the genus *Clunio* is not yet settled because distinctive morphological features characteristic of the various geographical strains are lacking. Within *C. marinus,* which is widely distributed on the European coast of the Atlantic Ocean, five races were distinguished on the basis of the larval habitat and the timing of emergence (Neumann, 1976). Among these local races, the Baltic Sea population was somewhat different from other Atlantic populations: it inhabits the sublittoral zone and tolerates salinities as low as 4–6 ppt in some areas (Palmén and Lindeberg, 1959; Olander and Palmén, 1968). The differences in larval habitat and time of emergence tend to isolate these populations, even at the site where both are simultaneously distributed, so they might be divisible into two species (*e.g.,* see Heimbach, 1978).

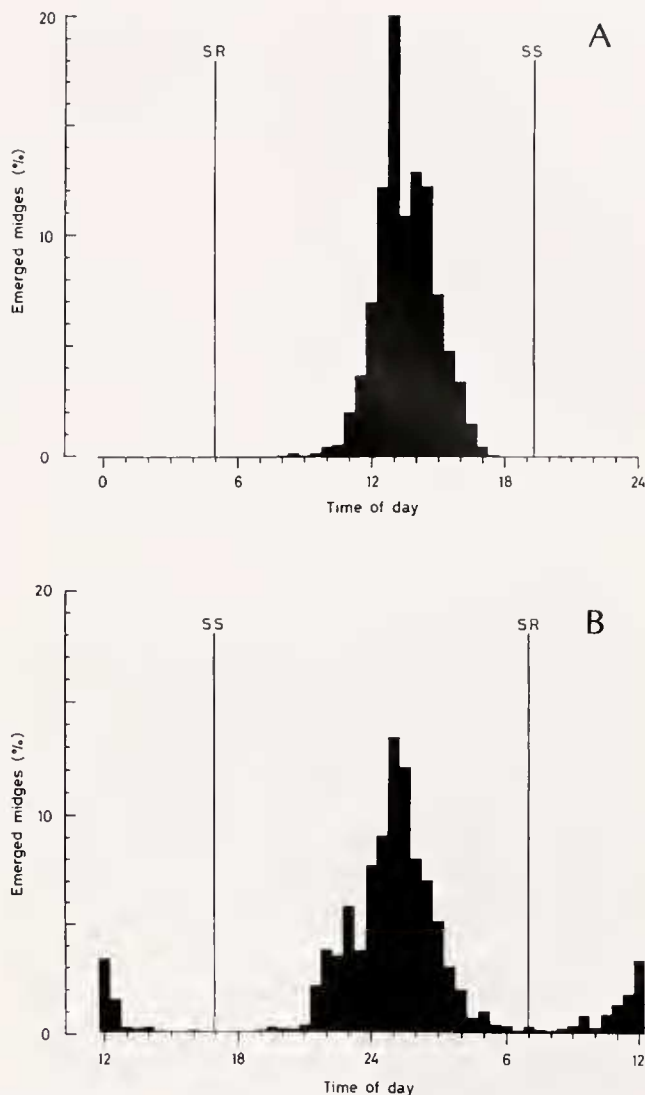Distributions of the antennal index, wing index, and body length (Fig. 2) all showed a single peak, and there

Figure 10. A pseudo-daily rhythm. (*A*) The time of emergence recorded from 17 June to 2 July 1991. (*B*) The time of emergence recorded from 28 November to 26 December 1991. The vertical axis shows the percentage of the midges collected. *SR* and *SS* indicate the times of sunrise and sunset, respectively. These times shift only a few minutes in June, and at most 15 min in December. In this figure, *SR* and *SS* show the times of sunrise and sunset on 17 June (A) and 28 November (B), respectively. See text for details.

was no difference between the population collected at the morning low tides and that collected at the afternoon low tides. This suggests that the midge *Clunio* inhabiting the seacoast of the Inland Sea is a single species. But which species? To determine whether the collected specimens are *C. tsushimensis* or *C. aquilonius,* the data in Figure 2 were compared with those obtained by Hashimoto (1968, 1969) in the Izu Peninsula, Shizuoka Prefecture. The antennal index (AI) of the individuals collected in March (Fig. 2A) was between 0.60 and 1.12, and the mean

was 0.84, intermediate between the Izu populations of *C. tsushimensis* (mean 1.12) and *C. aquilonius* (mean 0.62) collected in the same month. Moreover, the wing index (WI) of the Inland Sea species (Fig. 2B) ranged from 0.40 to 0.56, about the same as the mean for both *C. tsushimensis* and *C. aquilonius* at the Izu Peninsula. The body length (Fig. 2C) was between 2.00 and 3.10 mm (mean 2.40 mm), much larger than for *C. tsushimensis* from Izu collected during the same month (1.4–2.4 mm; mean about 1.8 mm). In summary, none of these indices could provide a positive identification of either *C. tsushimensis* or *C. aquilonius* at our observation site.

In the marine midges studied by Hashimoto (1969, 1976), the AI fluctuates with the season, with a minimum in early spring, a maximum in late summer, and no overlap between *C. tsushimensis* and *C. aquilonius* in any season; that is, whereas the AI (mean) of *C. aquilonius* varied from 0.6 in winter to 0.75 in summer, that (mean) of *C. tsushimensis* varied from 1.05 in winter to 1.4 in summer (see Fig. 7 in Hashimoto, 1969). The body length was also closely correlated with the annual change of water temperature (Hashimoto, 1976). Now, the atmospheric and water temperatures on the coast of the Inland Sea are 5–6°C lower in winter and spring than those on the Izu Peninsula. We therefore speculate that the decreased AI and increased body length are due to the temperature differential between the two larval habitats. Hence, we conclude that the species reported here is a local population of *C. tsushimensis*, and not a new species.

Acknowledgments

Literature Cited

Akiyama, T., and M. Yoshida. 1990.   The nocturnal emergence activity rhythm in the cumacean *Dimorphostylis asiatica* (Crustacea). *Biol. Bull.* 179: 178–182.

Caspers, H. 1951.   Rhythmische Erscheinungen in der Fortpflanzung von *Clunio marinus* (Dipt. Chiron.) und das Problem der lunaren Periodizität bei Organismen. *Archiv. Hydrobiol. Suppl.* 18: 415–594 (plus 4 photograph pages).

DeCoursey, P. J. 1983.   Biological timing. Pp. 107–162 in *The Biology of Crustacea VII Behavior and Ecology*. F. J. Vernberg and W. B. Vernberg, eds. Academic Press. Washington.

Enright, J. T. 1962.   Pressure sensitivity of an amphipod. *Science* 133: 758–760.

Enright, J. T. 1963.   The tidal rhythm of activity of a sand-beach amphipod. *Zeit. Vergl. Physiol.* 46: 276–313.

Enright, J. T. 1965.   Entrainment of a tidal rhythm. *Science* 147: 864–867.

Enright, J. T. 1972.   A virtuoso isopod: circa-lunar rhythms and their tidal fine structure. *J. Comp. Physiol.* 77: 141–162.

Hashimoto, H. 1968.   Seasonal variation in the marine chironomid, *Clunio tsushimensis* Tokunaga (Diptera). *Ann. Zool. Fenn.* 5: 41–48.

Hashimoto, H. 1969.   Affinity and differentiation of species in the marine chironomids, *Clunio aquilonius* and *C. tsushimensis* (Diptera). *Jpn. J. Zool.* 16: 19–45.

Hashimoto, H. 1976.   Non-biting midges of marine habitats (Diptera: Chironomidae). Pp. 377–414 in *Marine Insects*, L. Cheng, ed. North-Holland Publishing Company, Amsterdam.

Hauenschild, C. 1960.   Lunar periodicity. Pp. 491–497 in *Biological Clocks. Cold Spring Harbor Symposia on Quantitative Biology*, Vol. 25.

Heimbach, F. 1978.   Sympatric species, *Clunio marinus* Hal. and *C. balticus* n. sp. (Dipt., Chironomidae), isolated by differences in diel emergence time. *Oecologia* 32: 195–202.

Korringa, P. 1947.   Relations between the moon and periodicity in the breeding of marine animals. *Ecol. Monogr.* 17: 349–381.

Koskinen, R. 1968.   Seasonal emergence of *Clunio marinus* Haliday (Dipt., Chironomidae) in western Norway. *Ann. Zool. Fenn.* 5: 71–75.

Morgan, E. 1965.   The activity rhythm of the amphipod *Corophium volutator* (Pallas) and its possible relationship to changes in hydrostatic pressure associated with the tides. *J. Anim. Ecol.* 34: 731–746.

Neumann, D. 1966.   Die lunare und tägliche Schlüpfperiodik der Mücke *Clunio*. Steuerung und Abstimmung auf de Gezeitenperiodik. *Zeit. Vergl. Physiol.* 53: 1–61.

Neumann, D. 1976.   Adaptation of chironomids to intertidal environments. *Annu. Rev. Entomol.* 21: 387–414.

Neumann, D. 1985.   Circadian range of entrainment in the semilunar eclosion rhythm of the marine insect *Clunio marinus*. *J. Insect Physiol.* 31: 549–557.

Neumann, D. 1987.   Tidal and lunar rhythmic adaptations of reproductive activities in invertebrate species. Pp. 152–170 in *Comparative Physiology of Environmental Adaptation: Tide, Moon, Magnetic Field and Rhythmic Functions, Vol 3*, P. Pevet, ed., S. Karger, Basel.

Oka, H., and H. Hashimoto. 1959.   Lunare Periodizität in der Fortpflanzung einer pazifischen Art von *Clunio* (Diptera, Chironomidae). *Biol. Zentralblatt* 78: 545–559.

Olander, R., and E. Palmén. 1968.   Taxonomy, ecology and behaviour of the northern Baltic *Clunio marinus* Halid. (Dipt., Chironomidae). *Ann. Zool. Fenn.* 5: 97–110.

Palmén, E., and B. Lindeberg. 1959.   The marine midge, *Clunio marinus* Hal. (Dipt., Chironomidae), found in brackish water in the northern Baltic. *Int. Rev. Gesam. Hydrobiol. Hydrogr.* 44: 384–394.

Pearse, J. S. 1990.   Lunar reproductive rhythms in marine invertebrates: maximizing fertilization? Pp. 311–316 in *Advances in Invertebrate Reproduction 5*, M. Hoshi and O. Yamashita, eds. Elsevier Science Publishers B.V., London.

Saigusa, M. 1985.   Tidal timing of larval release activity in non-tidal environment. *Jpn. J. Ecol.* 35: 243–251.

Saigusa, M. 1986.   The circa-tidal rhythm of larval release in the incubating crab *Sesarma*. *J. Comp. Physiol. A* 159: 21–31.

Saigusa, M. 1988.   Entrainment of tidal and semilunar rhythms by artificial moonlight cycles. *Biol. Bull.* 174: 126–138.

Saigusa, M. 1992.   Phase shift of a tidal rhythm by light-dark cycles in the semi-terrestrial crab *Sesarma pictum*. *Biol. Bull.* 182: 257–264.